PERIODICITY OF MITOSIS AND CELL DIVISION IN THE EUGLENINEAE [1]

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In the course of an investigation into the division cytology of flagellates of the class Euglenineae, it became necessary to determine the time and rate of mitosis for each of the forty species under examination. The present paper deals with the periodicity of mitosis revealed in the twenty species studied in detail for this feature, and relationship of the periodicity to the day-night cycle. An account of the structure and division of the cell and nucleus will be published separately (see Leedale, 1958a, 1958b).

Material and Methods

1. *Species studied* [2]

The three main sources of material have been my own wild collections, the Cambridge Culture Collection of Algae and Protozoa, and the Sammlung von Algenkulturen, Göttingen. All species have been isolated by Professor E. G. Pringsheim or myself, with the exception of *Trachelomonas grandis* which was isolated by Singh (Singh, 1956) and sent to me by Professor H. C. Bold.

The names of species are corrected according to Pringsheim (1956) for the genus *Euglena*, and to Huber-Pestalozzi (1955) for the remaining genera. Colorless species are indicated by an asterisk.

* *Astasia klebsii* Lemmermann
Colacium mucronatum Bourrelly
Cryptoglena pigra Ehrenberg
* *Distigma proteus* Ehrenberg em. Pringsheim
Euglena acus Ehrenberg
Euglena deses Ehrenberg
Euglena gracilis Klebs (strain "T," green form)
* *Euglena gracilis* Klebs (strain "T," colorless form)
Euglena gracilis Klebs (strain "Z," green)
Euglena spirogyra Ehrenberg
Euglena viridis Ehrenberg
Eutreptia pertyi Pringsheim
Eutreptia viridis Perty

[1] From a study carried out in the Botany Departments of Queen Mary College, London, and The Durham Colleges; some of the results were included in a thesis approved for the degree of Doctor of Philosophy in the University of London. My thanks are due to Dr. M. B. E. Godward of Queen Mary College and Professor E. G. Pringsheim of the University of Göttingen for their help and advice.

[2] I would like to thank Professor E. G. Pringsheim, Mr. E. A. George of Cambridge and Professor H. C. Bold for supplying me with material.

* *Hyalophacus ocellatus* Pringsheim
Lepocinclis ovum var. *buetschlii* (Conrad) Huber-Pestalozzi
Lepocinclis steinii Lemmermann em. Conrad
* *Menoidium cultellus* Pringsheim
* *Peranema trichophorum* (Ehrenberg) Stein
Phacus pusillus Lemmermann
Phacus pyrum (Ehrenberg) Stein
Trachelomonas bulla Stein em. Deflandre
Trachelomonas grandis Singh

2. *Cultivation*

Cells were isolated from wild collections by the micropipette method (Pringsheim, 1946a). All species except *Peranema trichophorum* were grown in soil-water tubes (biphasic culture, Pringsheim, 1946a, 1946b) with a wheat grain, starch or ammonium magnesium phosphate beneath the soil. *Eutreptia* spp. were grown in tubes with 50% sea-water. *Peranema trichophorum* was grown in soil extract containing 0.5% milk.

In addition to the biphasic cultures, green and colorless forms of *Euglena gracilis* (strain "T") were cultivated in 0.2% Difco beef extract, or "SATBY" (0.1% sodium acetate, 0.2% Difco tryptone, 0.1% Difco beef extract, 0.2% Difco yeast extract, in distilled water).

Cultures were hung in a north-facing window or in temperature-controlled cabinets with either incandescent or fluorescent lighting on a time-switch. The cultures were grown at a standard temperature of 20° C.

General Features of the Cultures

A biphasic culture of any species of the Euglenineae has a typical growth pattern. Sub-culturing to a new tube with a heavy inoculum is followed by a lag-period of two to three days during which time there are few or no divisions. This is followed by a period of multiplication which is eventually slowed and halted by overcrowding of the medium. There is an upper limit of number of cells per ml. of medium (the "culture saturation point") at which cell multiplication falls to a low rate. This effect is not caused by exhaustion of the medium; if the cells of a "saturated" culture are centrifuged off and the medium re-inoculated, the culture builds up as quickly as before, and this can be repeated several times.

According to the size of the inoculum, the division rate, and the "culture saturation point" of the species concerned, the increase in cell numbers may continue for one to twelve months. It is the mitotic rhythms occurring during this period of multiplication which are the subject of this paper.

Mitotic Periodicity in Green Species

Fixations made at two-hourly intervals for several (not successive) 24-hour periods showed that all green species of the Euglenineae had mitosis confined to the dark period when growing in biphasic culture under natural light conditions.

The restriction of nuclear division to the dark period was examined in detail in

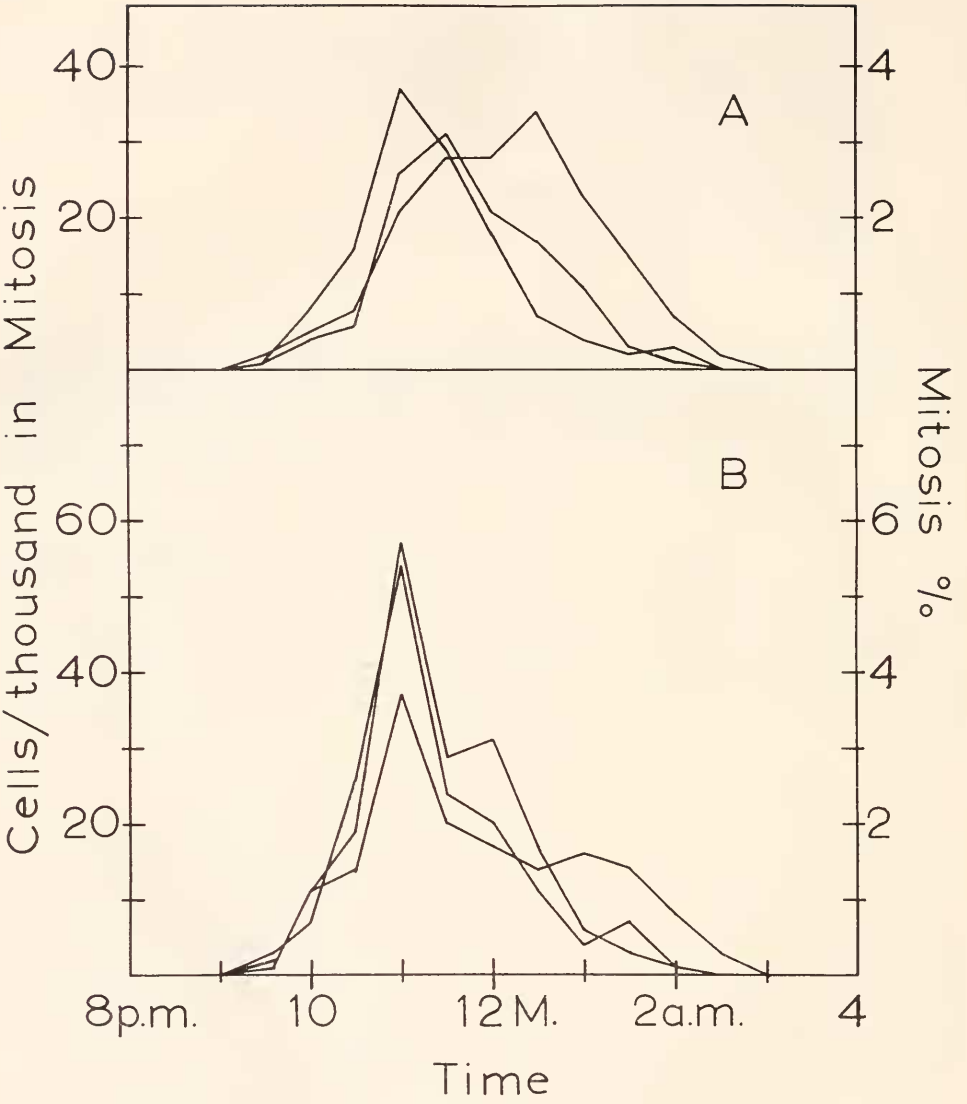
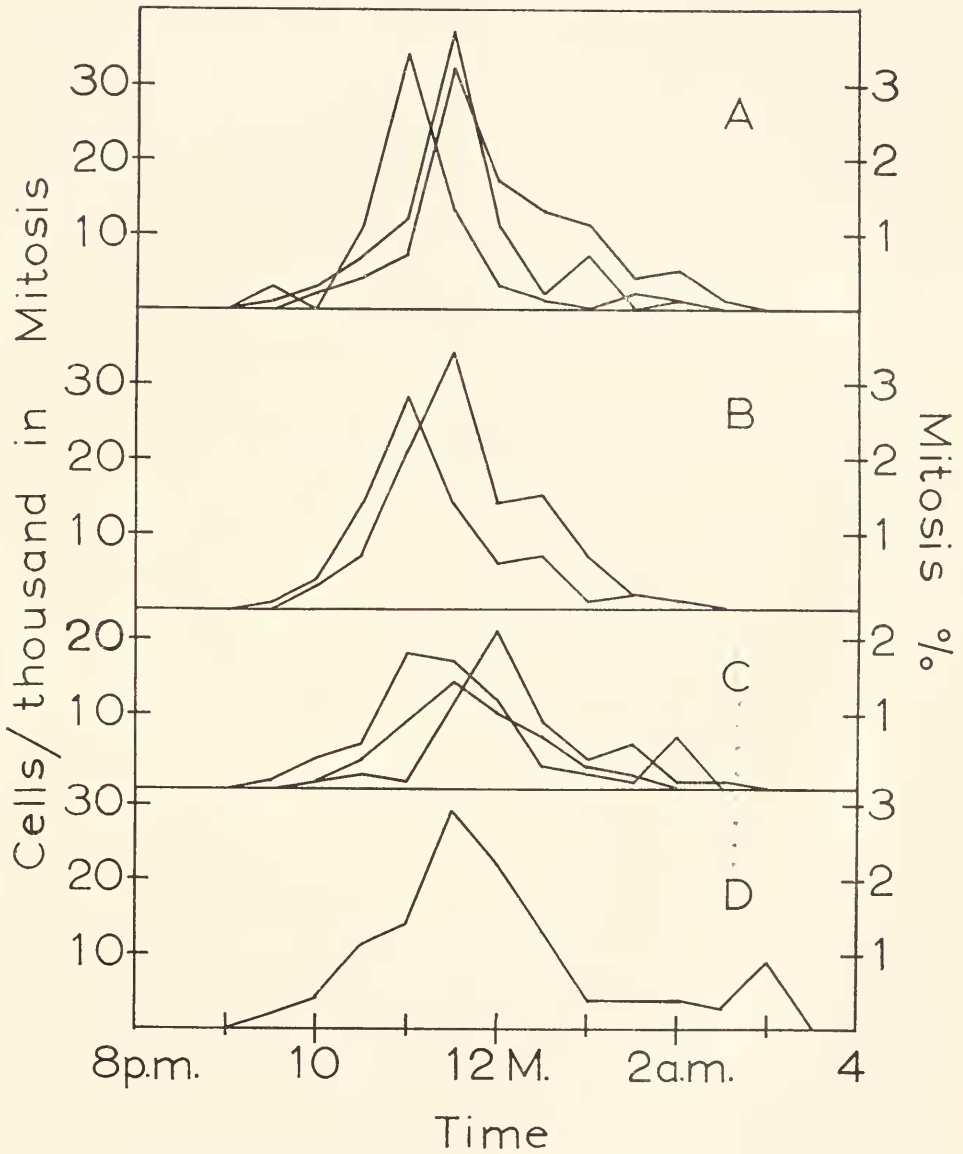


FIGURE 1.

fifteen green species. Fixations were made at half-hourly intervals from the onset of darkness on one, two or three consecutive nights, the material for any one series being taken from the same culture tube. Five hundred cells were counted in each of two preparations from each fixation and the number of cells in mitosis and cell division noted. The results of these counts were similar for all species and are recorded graphically for six representative species in Figures 1 and 2.

Mitosis began one to two hours after the onset of darkness. In *Euglena spirogyra* (Fig. 1, A), *Euglena viridis* (Fig. 1, B) and *Eutreptia pertyi,* mitosis began at the same time on each of three successive nights. The mitotic maximum



FIGURES 1 and 2. The number of cells per thousand in mitosis at half-hourly intervals during one, two or three consecutive nights, plotted as mitosis percentage against time. All results are for biphasic cultures growing in the natural day-night cycle, the dark period beginning at 8 PM. FIGURE 1. Green species: A, *Euglena spirogyra;* B, *Euglena viridis.* FIGURE 2. Green species: A, *Phacus pusillus;* B, *Phacus pyrum;* C, *Trachelomonas bulla;* D, *Trachelomonas grandis.*

occurred from 2½ to 4½ hours after the onset of darkness in all species. The maxima for *Euglena viridis* (Fig. 1, B), *Eutreptia pertyi* and *Phacus pusillus* (Fig. 2, A) occurred at the same time on three successive nights; those for *Euglena spirogyra* (Fig. 1, A) covered a two-hour period within three nights. The span

of the nightly period during which mitosis occurred ranged from three to six hours in the different species. The mean maximum percentage of cells undergoing mitosis each night is recorded in Table I.

Recording the number of cells at each stage of mitosis in each fixation produced a more detailed picture of the periodicity. The results for *Euglena spirogyra* for one dark period (Fig. 3) illustrate the complete restriction of nuclear and cell division to within a five-hour period, beginning approximately two hours after the onset of darkness. Successive maxima of the mitotic stages occur, a wave of prophases being followed by waves of metaphases, anaphases, telophases and cell cleavage. This pattern was repeated in other cultures of the same species and by other species, the relative size and span of the maxima varying according to the duration of the stages of mitosis in the different species.

TABLE I

The mean maximum percentage of cells undergoing mitosis each night in green species of the Euglenineae in biphasic culture at 20° C.

Species	Mean maximum %
Colacium mucronatum	2.6
Cryptoglena pigra	1.8
Euglena acus	1.9
Euglena deses	2.2
Euglena gracilis "T"	4.2
Euglena gracilis "Z"	5.7
Euglena spirogyra	3.4
Euglena viridis	4.9
Eutreptia pertyi	3.5
Eutreptia viridis	2.3
Lepocinclis ovum var. *buetschlii*	6.8
Lepocinclis steinii	1.3
Phacus pusillus	3.4
Phacus pyrum	3.1
Trachelomonas bulla	1.8
Trachelomonas grandis	2.9

Further series of fixations over a period of one year showed that no matter at what time of the clock the natural dark period began, mitosis began one to two hours later, the percentage of cells dividing each night being of the same order for any one species (at 20° C.). There was no variation in the mitotic rate in relation to day-length.

Examination of the same culture over a period of several months showed the multiplication period to be discontinuous. Weeks with divisions occurring every night were interspersed with occasional days when no divisions occurred.

The introduction of an artificial dark period during the natural light period affected mitotic periodicity in all the green species. If the artificial dark period was begun three hours or less before the natural one, mitosis occurred, but in a lower percentage of cells than usual. When the artificial dark period was introduced six hours or more before the natural one was due to begin, divisions rarely occurred. The shortest day-length after which mitosis would occur was approximately twelve hours.

No mitosis or cell division could be induced in any green species in biphasic

culture in any conditions or intensity of artificial lighting, either direct or diffused, incandescent or fluorescent. Attempts to reverse the mitotic periodicity in a temperature-controlled cabinet with lighting on a time-switch were unsuccessful, the cells becoming quiescent with no divisions occurring. Similarly, no mitosis occurred in either continuous light or continuous darkness. Returned to natural light conditions after such treatment, the cells recovered their full division rate within a day if the treatment had been short, but less quickly if the treatment was prolonged.

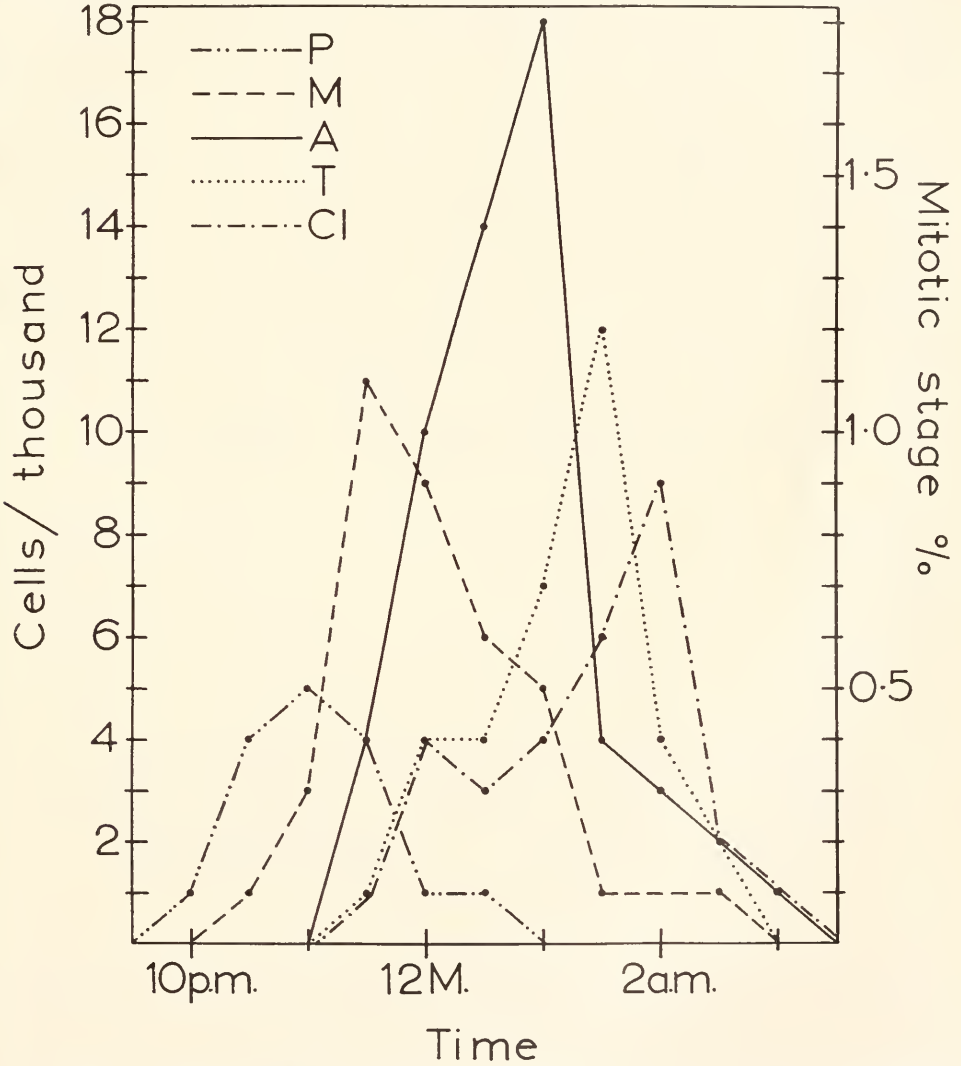


FIGURE 3. Mitosis in *Euglena spirogyra*. The number of cells per thousand in prophase (P), metaphase (M), anaphase (A), telophase (T) and cell cleavage (Cl) at half-hourly intervals during one night, plotted as percentage of each mitotic stage against time. The results are for a biphasic culture growing in the natural day-night cycle, the dark period beginning at 8 PM.

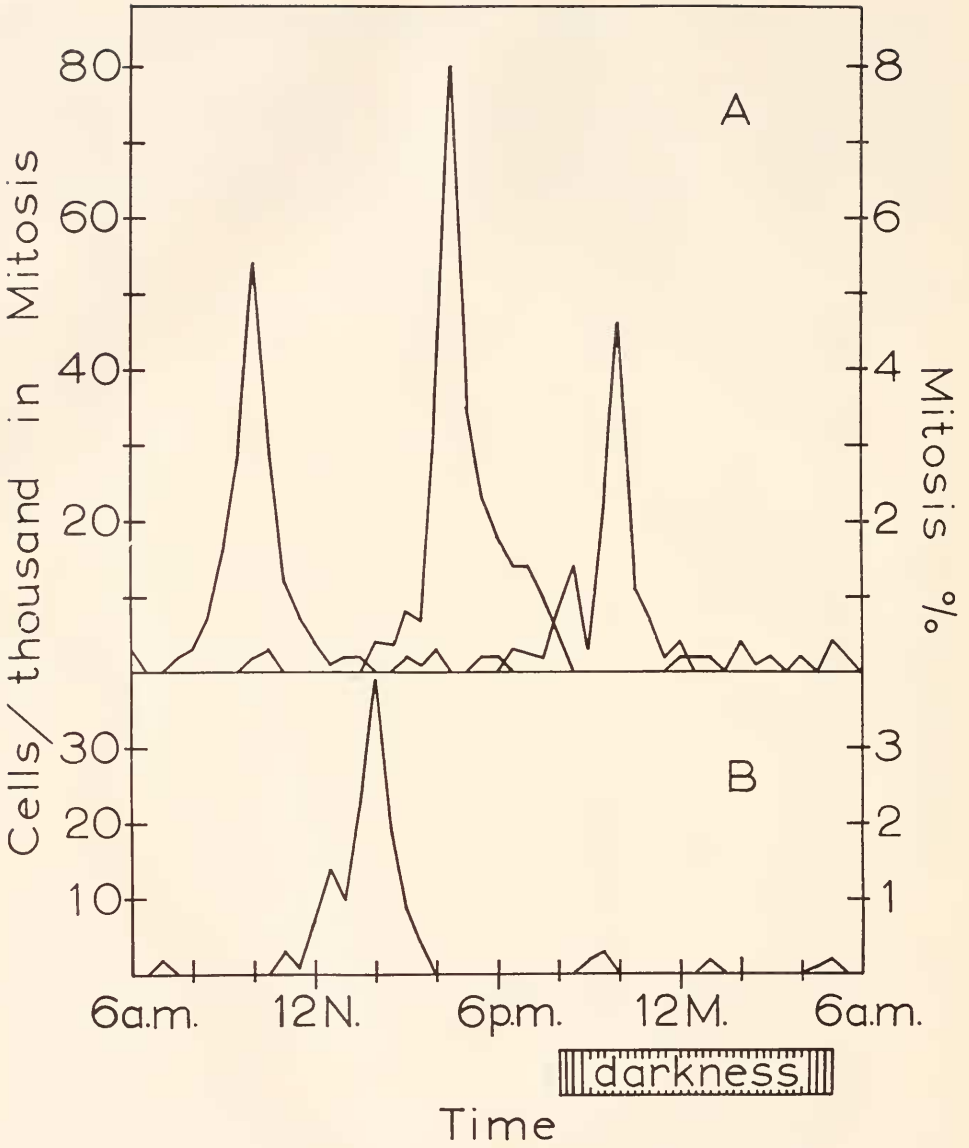
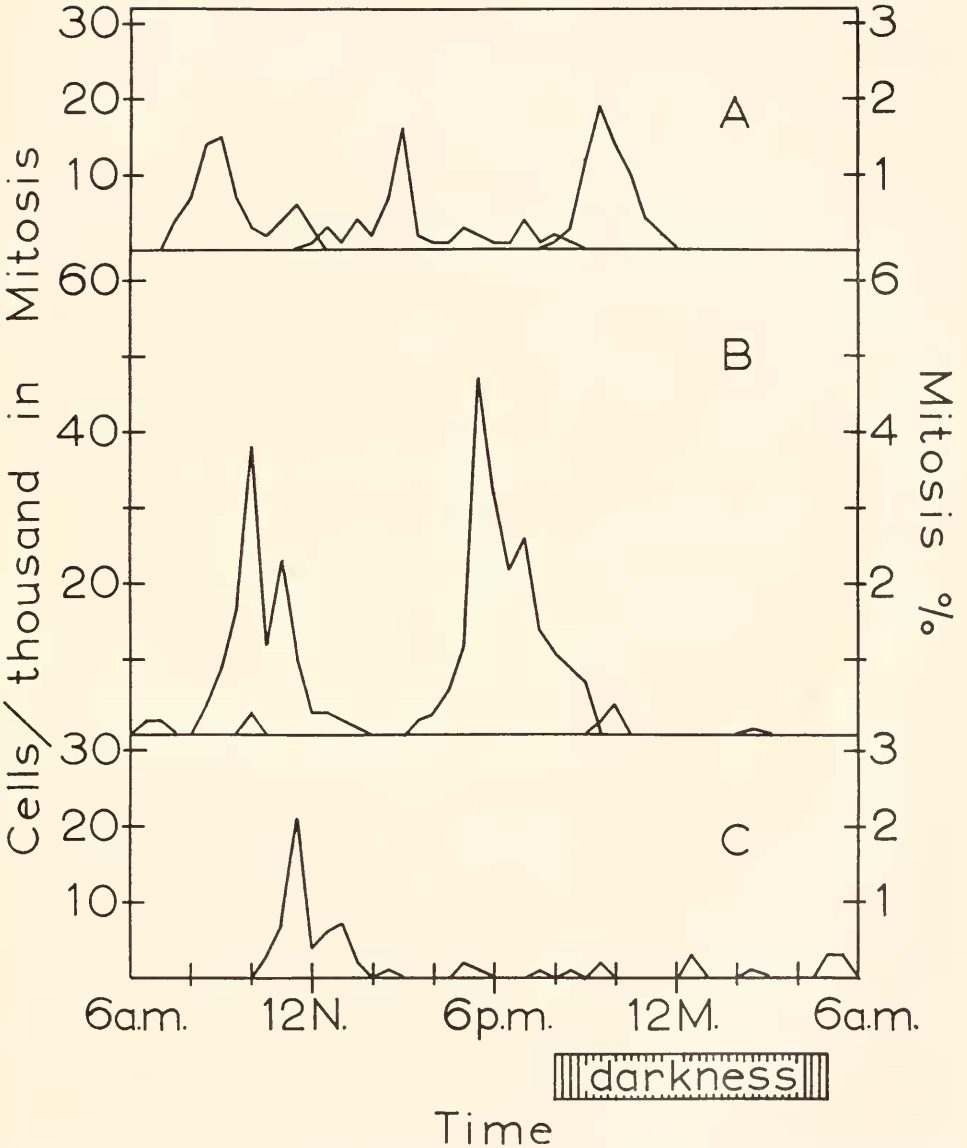


FIGURE 4.

Once mitosis had begun, it proceeded to conclusion even if the dividing cell was then subjected to light. However, if light was introduced less than an hour after the onset of darkness, no mitosis occurred. If a dark period of more than one hour followed a full-length day and artificial light was then introduced, some cells underwent a complete mitotic division, though on first examination *no cells could be found in mitosis, not even in prophase.*

Euglena gracilis was the only species in which the time and rate of mitosis in biphasic culture could be compared with those in a rich liquid medium. The



FIGURES 4 and 5. The number of cells per thousand in mitosis at half-hourly intervals during one, two or three (not consecutive) 24-hour periods, plotted as mitosis percentage against time. All results are for biphasic or milk cultures growing in the natural day-night cycle. FIGURE 4. Colorless species: A, *Astasia klebsii;* B, *Distigma proteus.* FIGURE 5. Colorless species: A, *Hyalophacus ocellatus;* B, *Menoidium cultellus;* C, *Peranema trichophorum.*

mitotic rhythm shown by the green form of strain "T" in biphasic culture was absent in 0.2% beef extract or "SATBY" medium. During the period of rapid multiplication prior to crowding of the culture, a fixation at any time of day or night showed from 5–6% (beef extract) or 8–10% ("SATBY") of the cells undergoing mitosis

(at 20° C.). At 30° C. the mitotic rate of *Euglena gracilis* "T" in "SATBY" was 25–30%. In biphasic culture, maximum division rates were obtained at 20° C.; raising or lowering the temperature by five degrees resulted in a fall in division rate.

Mitotic Periodicity in Colorless Species

Fixations made at half-hourly intervals over 24-hour periods showed that a constant rate of mitosis was not maintained in any colorless species of the Euglenineae in biphasic culture, bursts of mitotic activity alternating with periods when mitosis was almost completely absent.

The results for 24-hour series of half-hourly fixations are recorded for the five species studied in Figures 4 and 5. In addition to these series where a division maximum occurred at some time during the 24-hour period, numerous series contained no divisions or a few divisions scattered throughout the period. Many single fixations at different times of day or night contained cells in mitosis.

Table II

The maximum percentage of cells recorded in mitosis at any one time in colorless species of the Euglenineae in biphasic or milk culture at 20° C.

Species	Maximum %
Astasia klebsii	8.0
Distigma proteus	3.9
Hyalophacus ocellatus	1.9
Menoidium cultellus	4.7
Peranema trichophorum (in milk)	2.1

Mitotic maxima occurred at any time of the clock. In none of the five species did the periods of major mitotic activity bear any relationship to the alternating light and darkness of the natural day-night cycle. The recorded maxima for *Astasia klebsii* (Fig. 4, A) occurred at 10 AM, 4:30 PM and 10 PM; those for *Hyalophacus ocellatus* (Fig. 5, A) at 9 AM, 3 PM and 9:30 PM. The time-spans of the major periods of mitotic activity ranged from 3½ to 8½ hours.

The highest percentage of cells obtained dividing at any one time is recorded for each species in Table II. The percentages of cells dividing at different times on different dates were of the same order for some species (Fig. 5, A and B) but not for *Astasia klebsii* (Fig. 4, A).

The irregularly spaced bursts of major mitotic activity in the colorless species continued in alternating artificial light and darkness, in continuous light, and in continuous darkness.

The colorless form of *Euglena gracilis* "T" growing in 0.2% beef extract or "SATBY" medium behaved as did the green form in these media, exhibiting no periodicity of mitosis, regular or irregular. A continuous division rate of 6–7% was maintained in "SATBY" at 20° C., the rate increasing to 30–35% at 30° C.

Discussion

Mitotic rhythms have been recorded for higher plants by Lewis (1901), Kellicott (1904), Karsten (1915), Laughlin (1919), Stalfelt (1919), Friesner (1920), Tischler (1921), Abele (1925), Brown (1951) and Jensen and Kavaljian (1958).

The rhythm, in most cases thought to be endogenous, has been related to the onset of germination, the balance between cell elongation and division, or light periodicity. Lewis (1901) and Karsten (1915) found that the times of the maxima altered when light conditions were changed, but Friesner (1920) found the maxima were independent of light changes. Stalfelt (1919) and Brown (1951) state that the mitotic rhythm of higher plants is exogenously imposed by the day-night cycle, disappearing when the plants are grown in continuous darkness. No evidence of mitotic rhythms in higher plants was found by Winter (1929) or Gray and Scholes (1951).

Mitotic rhythms in animals have been recorded by Ortiz-Picon (1933), Carleton, (1934), Cooper and Schiff (1938), Cooper and Franklin (1940), Blumenfeld (1942, 1943), Bullough (1948) and Milletti (1950). The rhythm has been related to the activity cycle, a higher division rate occurring when the animal is at rest (Cooper and Schiff, 1938; Bullough, 1948; Milletti, 1950). Kalmus (1935) has recorded an exogenous rhythm of cell division for *Paramecium*.

Twenty-four-hour rhythms of mitosis have been recorded for a number of algae. Division occurring exclusively at night has been recorded for species of the genera *Cladophora* and *Stigeoclonium* (Braun, 1851), *Spirogyra* (Braun, 1851; Famintzin, 1867; Sachs, 1874; Strasburger, 1880), *Zygnema* (Kurssanow, 1912), and *Vaucheria, Hydrodictyon* and *Ulothrix* (Sachs, 1874), whilst Karsten (1918) found three maxima in each 24-hour period for species of *Closterium, Cosmarium* and *Mesotaenium*. Wildeman (1891) found no mitotic rhythm in *Spirogyra*. The present author has found mitosis almost entirely confined to the dark period in species of *Hydrodictyon, Ulothrix, Mougeotia, Spirogyra, Zygnema, Closterium, Cosmarium* and *Staurastrum* in biphasic culture. The rhythm was exogenous in these species, and mitosis could be produced at any time of the clock by adjusting the time of the dark period in a culture cabinet. Some species of *Spirogyra* and *Zygnema* underwent mitosis in continuous light.

A nocturnal periodicity of mitosis in euglenoid species has been mentioned by Dangeard (1902) for species of *Euglena, Phacus* and *Trachelomonas*, Baker (1926) for *Euglena gracilis* in a split pea infusion, Ratcliffe (1927) for *Euglena spirogyra* in modified Doflein's medium, S. R. Hall (1931) for the parasitic *Euglena leucops* Hall when in its host, a species of *Stenostomum*, Gojdics (1934) for *Euglena deses* in 0.1% beef extract, Johnson (1934) for *Colacium vesiculosum* Ehrbg. and Chu (1946) for *Euglena* spp. in biphasic culture. Only sparse growth was possible in several of the media recommended by these authors. Lackey (1929) has made the only record of a division maximum at night in a colorless species (*Entosiphon sulcatum* (Duj.) Stein, grown in a cracked wheat medium) and suggests it might be explained on phylogenetic grounds. This is to be doubted since the nocturnal rhythm of the green species is not endogenous and no such rhythm is present in the five colorless species investigated in the present study. Lackey records some divisions during the day and it is probable that his division maxima occurred during the dark period by chance, without being related to it.

The mitotic maxima recorded for higher plants and animals are *increases* over a continuous low division rate. As would be expected, in organisms composed of many cells arranged in tissues, some of which are specifically concerned with cell division, mitosis occurs throughout the 24-hour period in the division sites. Diurnal rhythms, whether in areas directly affected by light or not (root-tip meristems in

plants, bone marrow in animals), can be related to metabolic rhythms, maximum mitosis occurring during the period of minimum activity.

In unicellular organisms the division of labor between cell growth and mitosis is often in time rather than in space. A cell tends to divide during a period of minimum activity *of that particular cell.* Thus green unicells and filamentous green algae often have a rhythm of mitosis which is closely related to the rhythm of photosynthetic activity in the day-night cycle.

Such a relationship is exhibited by the Euglenineae. Green species, when living autotrophically, divide only in the dark, and an almost full period of natural daylight is necessary before mitosis will occur in the ensuing dark period. A threshold period of darkness is required for the induction of mitosis, but once induction has occurred, the mitotic process will begin and proceed to completion, even though the cell be subjected to light before its nucleus has begun the anterior migration which is the first sign of approaching mitosis. This induction precedes prophase by a period of up to one hour, since the threshold period for induction is approximately one hour after the onset of darkness, whilst the first prophases in all species appear one to two hours after darkness. The final inductions would then be occurring approximately three hours later, since the last prophases appear four to five hours after darkness (Fig. 3).

It has been shown in *Euglena gracilis* that the nocturnal periodicity of mitosis is removed by the stimulus of a rich food supply, the heterotrophic ("chemotrophic") mode of nutrition of this species in beef extract or "SATBY" being unrelated to the day-night cycle.

Mitosis in colorless species of the Euglenineae in biphasic culture shows an irregular periodicity which is not related to the natural day-night cycle. The heterotrophic mode of nutrition of the colorless species is also independent of light.

The factor deciding which cells in a culture divide during any one period of mitosis is probably cell age (reflecting cell size and cell maturity) in both green and colorless species. If 5% of the cells of a biphasic culture of a green species divide each night in turn, the span of a generation will be 20 days. In *Euglena gracilis* in "SATBY" at 30° C., with a division rate of 25–35%, the generation span cannot be more than 8–12 hours.

The nocturnal rhythm of mitosis is presumably present in green species in the wild when the supply of nutrients is low. An influx of rich organic nutrients will remove the periodicity and, when combined with optimum temperature and pH, may result in the sudden euglenoid "blooms" which often occur in bog-pools, farmyards, ponds and lakes.

Summary

1. The periodicity of mitosis and cell division has been investigated in 15 green and 5 colorless species of the Euglenineae.

2. Green species in biphasic culture under natural light conditions have mitosis confined to the dark period. Mitosis begins one to two hours after the onset of darkness, each species having a predictable percentage of cells dividing each night. There is a threshold period at the beginning of the dark period after which mitosis cannot be inhibited by light. The mitotic rhythm is exogenous, being removed by growth in artificial light or darkness (resulting in no mitosis), or in a rich organic medium (resulting in continuous mitosis at a constant rate).

3. Colorless species in biphasic culture under any light conditions have an irregular mitotic periodicity, bursts of mitosis occurring at any time of the clock and alternating with periods in which mitosis is almost absent.

4. It is suggested that the presence or absence of regular or irregular mitotic periodicity is related to the different modes and rates of nutrition of green and colorless species in various conditions of light and darkness, and in various media.

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